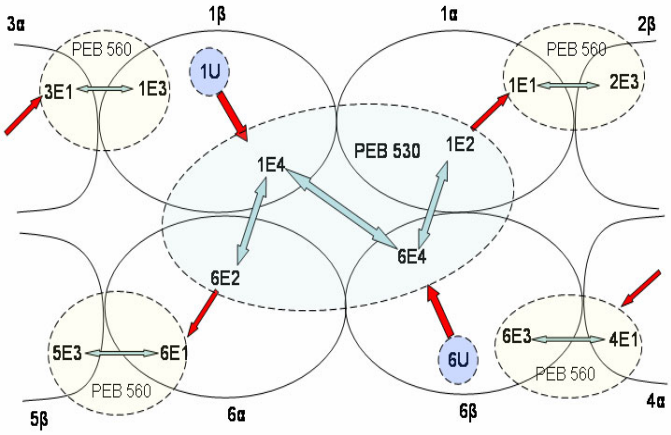
A Multistate Model for the Fluorescence Response of R-Phycoerythrin

R-phycoerythrin is a light-harvesting protein that absorbs over a broad range of wavelengths and channels the electromagnetic energy to a photoreactive center. R-PE and other phycobilin proteins, such as allophycocyanin (APC) and B-phycoerythrin (B-PE), are found in cyanobacteria and algae and have been studied extensively in the context of photosynthesis. Each 240 kDa R-PE molecule contains over 34 fluorophores that are linear or "open-chain" tetrapyrroles of two kinds, phycoerythrobilin (PEB) and phycourobilin(PUB). The fluorophores are attached to the protein backbone of R-PE via thioether linkages to conserved cysteine (cys) residues. When isolated from the photoreactive center, R-PE becomes highly fluorescent. As a result, these proteins have become popular fluorescent labels used extensively in biological assays.

A. Gaigalas, D.T. Gallagher, K.D. Cole, T. Singh and L. Wang (Div. 831), Y.-Z. Zhang, (Molecular Probes, Eugene, OR)

Although strong fluorescence makes the R-phycoerythrin (R-PE) proteins increasingly useful in biological and clinical assays, they are subject to nonlinear effects including exciton traps and photodegradation that complicate quantitative applications. We report measurements of R-PE fluorescence intensity as a function of incident power, duration of illumination, and temperature. Emission intensity in the primary band at 570 nm is proportional to incident power for low power levels. At higher incident power, the emission at 570 nm is lower than expected from a linear dependence on power. Our studies indicate the possibility that R-PE undergoes both reversible emission cessation on a millisecond time scale, attributed to transitions to nonradiative excited state (dark state), and irreversible photodegradation on a timescale of minutes. A multistate model, based on fluorescence measurements and geometric analysis, is thus proposed for the fluorophores in R-PE. The phycobilin fluorophores are partitioned into three groups: the phycourobilins (PUB) absorbing at 490 nm, one group of phycoerythobilins (PEB) absorbing at 530 nm (PEB-530), and another group of PEB absorbing at 560 nm (PEB-560). The two processes which result in the loss of fluorescence intensity are most likely associated with the PEB 560 group. In the future, investigations will be extended to the study of B-phycoerythrin, looking for general behavior. A proposed model of fluorophore groups and interactions in R-PE is shown below. The solid ovals represent α and β subunits. The six PUB, each one in a different β subunit, form a circular chain of weakly interacting fluorophores with an absorption maximum at 490 nm. The four strongly coupled fluorophores 1E2, 1E4, 6E2, and 6E4, each in a different subunit, form the PEB-530 unit. There are three such units distributed around the ring. Each pair of fluorophores (3E1, 1E3), (5E3,6E1), (1E1,2E3), and (6E3,4E1) form dimers that absorb strongly at 560 nm. There are six such dimers distributed around the R-PE ring. The various



groups of PEB are encircled by dashed ovals. The energy is transferred incoherently from PUB to PEB-530 and then to PEB-560. The fluorophores within the PEB-530 and PEB-560 groups form exciton states. Arrows indicate strong coupling interactions, as determined by short distances and dipole alignments.

Publication

A. Gaigalas, D.T. Gallagher, K.D. Cole, T. Singh, L. Wang, and Y.-Z. Zhang, **"A Multistate Model for the Fluorescence Response of R-Phycoerythrin"**. *Photochemistry and Photobiology*: in press, 2006.